Journal of Bioenergetics and Biomembranes, Vol. 24, No. 5, 1992

Minireview

Identification of Subunits Required for the Catalytic Activity of the F_1-ATPase

Zippora Gromet-Elhanan[1]

Received May 5, 1992; accepted May 20, 1992

$F_1(\alpha\beta)$ complexes containing equimolar ratios of the α and β subunits have been shown to function as active ATPases, whereas individually isolated α and β subunits show no real ATPase activity. These results indicate that the single-copy subunits are not required for F_1-ATPase activity. The minimal $F_1(\alpha\beta)$-core complexes exhibit, however, lower rates and some different properties from those of their parent whole F_1 or $\alpha_3\beta_3\gamma$ complexes. It is therefore concluded that for obtaining a full spectrum of the characteristic functional properties of an F_1-ATPase the presence of the F_1-γ subunit is also required. The implications of these findings on the subunit location of both catalytic and noncatalytic nucleotide binding sites is discussed.

KEY WORDS: F_1-ATPase; ATP synthase; catalytic subunits; nucleotide binding sites; cooperative kinetics; multisite catalysis; azide; tentoxin.

1. INTRODUCTION

The F_1-ATPase is the catalytic sector of the membrane-bound proton-translocating F_0F_1 ATP synthase–ATPase complex. It is an oligomeric complex composed of five different subunits with a stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ that is able to hydrolyze ATP in its isolated, soluble state. This complex structure hinders studies aimed at elucidating its catalytic site(s) and mechanism of action. Various methods have therefore been developed for the isolation of single subunits or partial complexes in their native, active state. They provide simpler experimental systems for detailed studies of the number and properties of substrate binding sites, characterization of catalytic activity, and reconstitution of minimal catalytic complexes.

This article summarizes recent studies on individual subunits and several partial complexes that have been isolated from membrane-bound F_1-ATPases of spinach chloroplasts and *R. rubrum* chromatophores. It also includes a brief comparison of their properties with those of similar preparations isolated from nonphotosynthetic aerobic bacteria. For fields that are not covered here and for more comprehensive reviews, the reader should refer to other minireviews in this issue and to recent review articles (Ysern *et al.*, 1988; Boyer, 1989; Futai *et al.*, 1989; Senior, 1990; Penefsky and Cross, 1991).

2. ISOLATION AND PROPERTIES OF INDIVIDUAL α AND β SUBUNITS

Isolation of native $F_1\alpha$ and/or β subunits has been achieved by different approaches. One involves partial or complete dissociation of the F_1 complex into its individual subunits. Complete dissociation followed by isolation and purification of either the major three or all five subunits was reported for EcF_1 from *E. coli* (Futai, 1977), TF_1 from thermophilic bacterium PS3 (Yoshida *et al.*, 1977), and StF_1 from *S. typhimurium* (Hsu *et al.*, 1984), respectively. With chloroplast CF_1 only a partial dissociation was achieved. It enabled the isolation of $CF_1\beta$ but not of $CF_1\alpha$ or $CF_1\gamma$ (Richter *et al.*, 1986).

The second approach involves selective removal, by incubation with LiCl followed by LiBr, of in-

[1]Department of Biochemistry, The Weizmann Institute of Science, Rehovot 76100, Israel.

0145-479X/92/1000-0447$06.50/0

dividual subunits or partial complexes from the membrane-bound F_1-ATPase. In *R. rubrum* chromatophores sequential removal, isolation, and purification of the $RrF_1\beta$ and γ subunits was achieved, leaving fully reconstitutable β-less and β,γ-less chromatophore membranes (Philosoph *et al.*, 1977; Khananshvili and Gromet-Elhanan, 1982). Incubation of thylakoids from various plants with LiCl in presence of MgATP resulted in a more complicated pattern of removed CF_1 subunits (Avital and Gromet-Elhanan, 1990; Avni *et al.*, 1991). Further fractionation of the spinach LiCl-extract led to isolation and purification of $CF_1\beta$ as well as of a $CF_1(\alpha\beta)$ complex containing equal amounts of the α and β subunits (Avital and Gromet-Elhanan, 1991; see Section 3 below).

The genetic approach developed in *E. coli* has recently been applied for the isolation of large quantities of individual F_1 subunits by cloning and overexpression of their genes (Futai *et al.*, 1988). This technique has already been used also for isolation of the major TF_1 α, β, and γ subunits (Ohta *et al.*, 1988). It is very promising especially since it enables the application of site-directed mutagenesis for studying the role of specific amino acid residues in substrate binding and catalytic activity.

Mitochondrial, bacterial, and recently also spinach chloroplast F_1-ATPases were found to have six nucleotide binding sites (Penefsky and Cross, 1991; Shapiro *et al.*, 1991) that reside in their α and β subunits. These findings together with their $\alpha_3\beta_3$ stoichiometry led to the suggestion that each of these subunits contains a single nucleotide-binding site. These sites were designated as catalytic or noncatalytic according to their exchangeability with medium nucleotides, and since nucleotide affinity probes were shown to label the β subunits of many F_1-ATPases under conditions that led to loss of activity, the catalytic sites were assumed to be located on the β subunits.

Direct binding studies with labeled ATP or ADP have indeed revealed the presence of a single Mg-independent nucleotide-binding site on the isolated $EcF_1\alpha$ (Dunn and Futai, 1980) and also on $EcF_1\beta$ (Issartel and Vignais, 1984). Similar direct labeling tests with subunits isolated from other sources yielded, however, different results. In $TF_1\alpha$ a single, but Mg-dependent, ADP-binding site was reported whereas in $TF_1\beta$ a single Mg-independent site was observed (Hisabori *et al.*, 1986). In isolated $RrF_1\beta$ such direct binding studies have identified *two* different binding sites for either ATP (Gromet-Elhanan and Khananshvili, 1984) or ADP (Khananshvili and Gromet-Elhanan, 1984), one being Mg-independent and the other Mg-dependent. These results questioned the validity of the proposed model of a single binding site on each α and β subunit. To accommodate these findings with the overall number of six nucleotide binding sites per F_1 molecule, one of the two nucleotide binding sites on the β subunit, the Mg-independent site, has been suggested to reside at an interface between the α and β subunits (Gromet-Elhanan and Khananshvili, 1984).

Further evidence for this suggestion came from ^{32}Pi binding tests, which revealed only one Mg-dependent Pi binding site on $RrF_1\beta$ (Khananshvili and Gromet-Elhanan, 1985a), and from the effect of chemical modification of $RrF_1\beta$ by diethyl pyrocarbonate and Woodward's reagent K (Khananshvili and Gromet-Elhanan, 1985b). Both reagents did not affect the binding of ADP to both sites on $RrF_1\beta$ nor its capacity to rebind to β-less chromatophores, but inhibited completely the Mg-dependent binding of Pi, the binding of ATP to its Mg-dependent site, and the capacity of the modified rebound $RrF_1\beta$ to restore ATP synthesis in the reconstituted chromatophores. These results have thus identified the Mg-dependent ATP and Pi binding site on $RrF_1\beta$ as the catalytic site and the Mg-independent site, which is located at the interface between the α and β subunits, as the noncatalytic one (Khananshvili and Gromet-Elhanan, 1985b). More recent studies on the photolysis of 2-azidoadenine nucleotides bound at either catalytic or noncatalytic sites on MF_1, EcF_1, and CF_1 have indicated that the β subunit was labeled in all cases (Boyer, 1987). These findings thus provide additional support for our model suggesting the presence of at least parts of the adenosine moiety of both nucleotide-binding sites on the $F_1\beta$ subunit and the possible sharing of one of them by both the α and β subunits (Futai *et al.*, 1989; Penefsky and Cross, 1991, see also Section 4 below).

All isolated, pure α and β subunits were reported to have either no measurable (Futai, 1977; Yoshida *et al.*, 1977; Philosoph *et al.*, 1977; Avital and Gromet-Elhanan, 1991) or very low ATPase activity, which required more than 10 hours of incubation for its assay (Roux-Framy *et al.*, 1987; Al-Shawi *et al.*, 1990). Even the $TF_1\alpha$ and β subunits, which were obtained by cloning and overexpression and found to be more active than those prepared from dissociated TF_1 (Kagawa *et al.*, 1989), were reported to have no meaningful ATPase activity (Miwa and Yoshida, 1989).

Also isolated purified $CF_1\beta$ containing about 5% of $CF_1\alpha$ showed no measurable ATPase activity (Richter *et al.*, 1986; Avital and Gromet-Elhanan, 1991). Furthermore, earlier reported higher ATPase activities in isolated $RrF_1\beta$ (Harris *et al.*, 1985) and $CF_1\beta$ (Frasch *et al.*, 1989) have been suggested as due to contamination by $RrF_1\alpha$ (Al-Shawi *et al.*, 1990) or by a non-CF_1-ATPase (Avital and Gromet-Elhanan, 1991).

The reported inability of all isolated $F_1\alpha$ and β subunits to catalyze ATP hydrolysis by themselves was not due to their isolation in an inactive form, since they were able to reconstitute either membrane-bound or soluble F_1-ATPase activity. Thus, isolated $RrF_1\beta$ was shown to reconstitute β-less *R. rubrum* chromatophores and restore their ATP synthesis and hydrolysis activities to the same extent (Philosoph *et al.*, 1977; Khananshvili and Gromet-Elhanan, 1982). Also pure $EcF_1\beta$ (Gromet-Elhanan *et al.*, 1985) and $CF_1\beta$ containing $\sim 5\%$ of $CF_1\alpha$ (Richter *et al.*, 1986; Avital and Gromet-Elhanan, 1991) were capable of reconstituting the β-less *R. rubrum* chromatophores and forming hybrid membrane-bound active F_1-ATPases. These hybrids could, however, catalyze only very low rates of ATP synthesis.

The isolated $F_1\alpha$ and β subunits of EcF_1 (Futai, 1977), TF_1 (Yoshida *et al.*, 1977), and StF_1 (Hsu *et al.*, 1984) were all found capable of reconstituting native or hybrid (Futai *et al.*, 1980; Hsu *et al.*, 1984) soluble F_1-ATPase activity when recombined in presence of the isolated $F_1\gamma$ subunits (see, however, Section 3).

3. ISOLATION AND PROPERTIES OF $F_1(\alpha\beta)$ COMPLEXES

Earlier experiments seemed to indicate that an asymmetric structure is a prerequisite for F_1-ATPase activity, since the minimal combination of isolated subunits reported to reconstitute a stable active ATPase was either $\alpha_3\beta_3\gamma$ or $\alpha_3\beta_3\delta$ (Futai, 1977; Yoshida *et al.*, 1977). In all cases, a tenfold lower ATPase activity was observed with reconstituted mixtures of only α and β subunits, but a stable $(\alpha\beta)$ complex has not been isolated.

Recently, however, active F_1-ATPase complexes containing only TF_1 or CF_1 α and β subunits have been isolated (Kagawa *et al.*, 1989; Miwa and Yoshida, 1989; Avital and Gromet-Elhanan, 1991). The $TF_1(\alpha\beta)$ complexes were prepared from a 1 : 1 mixture of individually overexpressed α and β subunits, which assemble in the absence of nucleotides and Mg^{2+} into an $\alpha_3\beta_3$ complex (Miwa and Yoshida, 1989; Kagawa *et al.*, 1989). In the presence of nucleotides and Mg this $\alpha_3\beta_3$ hexamer was shown to dissociate into $\alpha_1\beta_1$ dimers, which are also active ATPases (Harada *et al.*, 1991).

A direct assembly of chloroplast $CF_1\alpha$ and β subunits into active $CF_1(\alpha\beta)$ complexes is as yet impossible, since all attempts to isolate pure $CF_1\alpha$ in a soluble active form have failed (Jagendorf *et al.*, 1991). An assembled active $CF_1(\alpha\beta)$ preparation, containing an equimolar ratio of α and β subunits, was however isolated and purified from the spinach membrane-bound CF_0F_1. The procedure involves extraction of coupled spinach thylakoids with 2 M LiCl in presence of 4 mM MgATP (Avital and Gromet-Elhanan, 1990), followed by fractionation of the resulting extract on FPLC ion exchange columns (Avital and Gromet-Elhanan, 1991). In presence of MgATP the purified $CF_1(\alpha\beta)$-ATPase appears as a mixture of $\alpha_3\beta_3$ and $\alpha_1\beta_1$ but, unlike with the $TF_1(\alpha_3\beta_3)$, removal of MgATP leads to loss of ATPase activity (Sokolov, Avital and Gromet-Elhanan, unpublished results).

Such differences in the effect of nucleotides have earlier been observed when a $TF_1(\alpha_3\beta_3\gamma)$ ATPase complex was reconstituted from individual subunits in the complete absence of Mg and nucleotides (Yoshida *et al.*, 1977), whereas the assembly of a similar EcF_1 ATPase-complex was absolutely dependent on presence of MgATP (Dunn and Futai, 1980). These reported differences in stability of the partial complexes of TF_1, CF_1, and EcF_1 may be due to some intrinsic differences in their nucleotide-binding properties. Indeed, isolated TF_1, unlike all other mesophilic F_1-ATPases, has no endogenously bound nucleotides (Ohta *et al.*, 1980).

The reversible dissociation of the $TF_1(\alpha_3\beta_3)$ hexamer into its $\alpha_1\beta_1$ dimer in presence of MgAT(D)P, and the destabilization of the $CF_1(\alpha\beta)$ complexes by removal of MgATP, complicate at present studies aimed at comparing their nucleotide binding sites with those observed on their parent F_1-ATPases or on the isolated α and β subunits.

All isolated $F_1(\alpha\beta)$ complexes are capable of ATP hydrolysis at rates that vary between 0.4 to 0.8 units/mg for the $TF_1(\alpha\beta)$ complexes (Kagawa *et al.*, 1989; Miwa and Yoshida, 1989) and 0.05 to 0.2 units/mg for the $CF_1(\alpha\beta)$ complex (Avital and Gromet-Elhanan, 1991; Gromet-Elhanan and Avital, 1992). When assayed under identical conditions the activity of $TF_1(\alpha\beta)$ amounts to 10–20% of the activities of its native

parent TF_1 or the reconstituted $TF_1(\alpha_3\beta_3\gamma)$. Such direct comparison is not possible for the $CF_1(\alpha\beta)$ complex since the native parent CF_1 is a latent ATPase whose activation requires removal of its ε subunit and reduction of a disulfide bond in its γ subunit (McCarty and Richter, 1987). It is therefore not surprising that $CF_1(\alpha\beta)$ is by itself a more active MgATPase than its parent, latent CF_1. Its specific ATPase activity amounts, however, to about 1% of that observed with a $CF_1(\alpha\beta\gamma)$ prepared by stepwise removal of the δ and ε subunits from CF_1 (Patrie and McCarty, 1984). These $F_1(\alpha\beta)$ complexes thus represent the minimal catalytic core of their respective F_1-ATPases.

The catalytic properties reported to date for the $F_1(\alpha\beta)$ core complexes are in some respects similar but in others different from their parent F_1-ATPases (Miwa and Yoshida, 1989; Kagawa *et al.*, 1989; Gromet-Elhanan and Avital, 1992). The most interesting differences involve the effects of inhibitors and stimulators such as tentoxin and azide. Tentoxin is produced by a plant pathogenic fungus and functions as a specific effector of CF_1-ATPases from sensitive plants, such as spinach or lettuce (Steele *et al.*, 1976), inhibiting them at $< 1\,\mu M$ but stimulating at $> 100\,\mu M$ (Steele *et al.*, 1978). Unlike the CF_1-ATPase the *R. rubrum* soluble and membrane-bound RrF_1-ATPase is resistant to even 1 mM tentoxin (Weiss and Gromet-Elhanan, unpublished results). This dramatic difference in sensitivity to tentoxin was earlier used to differentiate between the resistant native RrF_1-ATPase and the sensitive hybrid F_1-ATPase formed by reconstitution of isolated $CF_1\beta$ into β-less *R. rubrum* chromatophores (Richter *et al.*, 1986). The spinach $CF_1(\alpha\beta)$ core complex could bind tentoxin rather tightly but was only stimulated by it, whereas the membrane-bound hybrid F_1-ATPase, formed by reconstitution of the same preparation of $CF_1(\alpha\beta)$ into β-less chromatophores, was fully inhibited by tentoxin (Gromet-Elhanan and Avital, 1992). These results indicate that for stimulation by tentoxin the presence of α and/or β subunits from a sensitive CF_1 is enough, but for inhibition by it other F_1 subunits are required. Since in the hybrid all other F_1 subunits come from the tentoxin-resistant RrF_1, their role in inducing tentoxin inhibition must be indirect. An indication that the γ subunit is responsible for this indirect effect came from recent observations that spinach $CF_1(\text{-}\delta\text{-}\varepsilon)$ is fully inhibited by $3\,\mu M$ tentoxin (McCarty, personal communication).

Azide is a general potent inhibitor of membrane-bound and soluble F_1-ATPases, including TF_1 and CF_1. All isolated $F_1(\alpha\beta)$ core complexes show, however, no sensitivity to it (Kagawa *et al.*, 1989; Miwa and Yoshida, 1989; Gromet-Elhanan and Avital, 1992), whereas the hybrid F_1-ATPase formed with $CF_1(\alpha\beta)$ and β-less chromatophores was fully inhibited. Azide was reported to inhibit multisite, but not unisite, ATP hydrolysis by F_1, and was therefore suggested to block catalytic cooperativity (Futai *et al.*, 1989). The observation that all isolated $F_1(\alpha\beta)$ core complexes are insensitive to azide could therefore suggest that they lack the functional heterogeneity observed in F_1-ATPases. The $TF_1(\alpha_3\beta_3)$ complex was, however, shown to exhibit cooperative kinetics similar to those of its parent TF_1-ATPase (Miwa and Yoshida, 1989). But these results were obtained under conditions that are now known to induce the reversible dissociation of the $\alpha_3\beta_3$ hexamer into its $\alpha_1\beta_1$ dimers (Harada *et al.*, 1991). Since the dimers are also active ATPases, the interpretation of such kinetic measurements becomes very complicated.

The isolation of $F_1(\alpha\beta)$ complexes that are active ATPases, but have lower rates of catalysis than their parent F_1-ATPases and are resistant to inhibition by tentoxin and/or azide, suggests that the single copy F_1 subunits are not required for assembly of these F_1-core complexes nor for their activity. They are, however, responsible for the 10- to 100-fold increased rates and for the markedly different properties of the whole F_1-ATPases. The $F_1(\alpha\beta)$ complexes can therefore provide an important tool for elucidating the role of the single-copy subunits in the structure and function of the F_1-ATPase.

4. SUBUNIT LOCATION OF NUCLEOTIDE-BINDING SITES

A model for the gross overall location of both catalytic and noncatalytic nucleotide-binding sites on the α and β subunits of the F_1-ATPase is presented in Fig. 1. It differs from earlier models in depicting the catalytic as well as noncatalytic sites as being located at an interface between the α and β subunits, with a different orientation than that of adenylate kinase. The absence of a transphosphorylation reaction in F_1 (Penefsky and Cross, 1991) raises the possibility of structural differences between the nucleotide binding sites of both enzymes, although there are certainly conserved regions of amino acid sequences that participate in such sites in many different enzymes (Ysern *et al.*, 1988). The model presents one α and two β

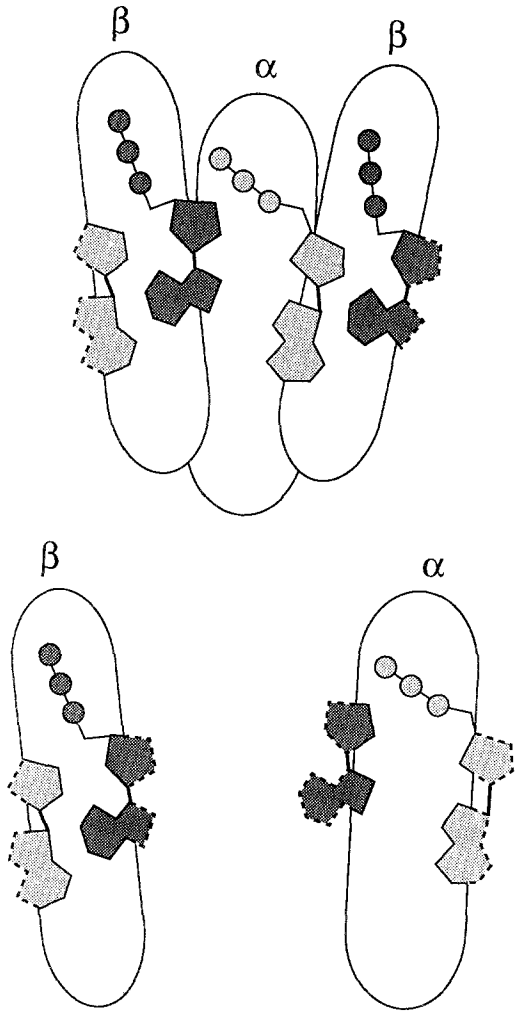

Fig. 1. A speculative model for the location of the catalytic (dark shaded, mainly on the β subunit) and noncatalytic (light shaded, mainly on the α subunit) nucleotide-binding sites on each isolated $F_1\alpha$ and β subunit and on a partially assembled F_1 complex.

subunits in the assembled state as elongated structures (Penefsky and Cross, 1991), and in light of the recently reported quaternary structure of the rat liver mitochondrial F_1 moiety (Bianchet *et al.*, 1991), the β subunits are protruding above the α subunit and can interact strongly with it, but not with each other.

The specific new features of this speculative model were designed as an attempt to explain a number of poorly understood reported observations (see Sections 2 and 3 above). Thus, the catalytic site is situated mainly on the β subunit and the noncatalytic one mainly on the α subunit, but neither subunit has a complete nucleotide-binding site, although each of them contains parts of both binding sites. This arrangement can explain why all isolated $F_1\beta$ subunits cannot hydrolyze ATP by themselves, but both an $\alpha_1\beta_1$ dimer and an $\alpha_3\beta_3$ hexamer can do it (Harada *et al.*, 1991). It also predicts that the binding constants of the partial binding sites, present on each isolated individual subunit, might differ from those recorded for the complete sites on the assembled F_1 complex, or even on assembled $F_1(\alpha\beta)$ complexes. Thus, individual subunits might be able to bind either zero or one or even two molecules of ATP or ADP (see Section 2 above), depending on the changes in the binding constants induced by the dissociation procedures.

The more detailed presentation of the binding sites remaining on the individual α and β subunits after their dissociation (Fig. 1) suggests that most of the catalytic site remains located on the β subunit and only a small part of its adenosine moiety appears on the isolated α subunit. On the other hand, the phosphate groups and a small part of the adenosine moiety of the noncatalytic binding site remain on the isolated α subunit, but most of the adenosine moiety of this site appears on the β subunit. This arrangement explains the findings that two ADP or ATP binding sites could be identified more easily on the isolated β than on the isolated α subunit, but only one Pi binding site was observed on isolated β (see Section 2 above). It also suggests that direct binding studies with ^{32}P might reveal one Pi binding site also on the α subunit.

The binding change mechanism requires that the three distinct catalytic sites located on the β subunits of F_1 communicate with each other via indirect conformational changes (Boyer, 1987, 1989). But it does not specify any function for the noncatalytic sites. If, as suggested by the quaternary structure of MF_1, the β subunits interact with adjacent α subunit but not with each other (Bianchet *et al.*, 1991), binding to the noncatalytic sites on α could play an important role in guiding the communication between the β subunits. Indeed, both in $EcF_1\alpha$ (Senda *et al.*, 1983) and in $RrF_1\beta$ (Khananshvili and Gromet-Elhanan, 1986), occupation of the nucleotide binding site(s) has been shown to induce large changes in conformation.

ACKNOWLEDGMENTS

This work was supported by grants from the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel, the Minerva Foundation, Munich, Germany, and the Basic Research Foundation

administered by the Israel Academy of Sciences and Humanities.

REFERENCES

Al-Shawi, M., Parsonage, D., and Senior, A. E. (1990). *J. Biol. Chem.* **265**, 5595–5601.

Avital, S., and Gromet-Elhanan, Z. (1990). In *Current Research in Photosynthesis* (Baltscheffsky, M., ed.), Vol III, Kluwer Academic Publishers, Dordrecht, pp. 45–48.

Avital, S., and Gromet-Elhanan, Z. (1991). *J. Biol. Chem.* **266**, 7067–7072.

Avni, A., Avital, S., and Gromet-Elhanan, Z. (1991). *J. Biol. Chem.* **266**, 7317–7320.

Bianchet, M., Ysern, X., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1991). *J. Biol. Chem.***266**, 21197–21201.

Boyer, P. D. (1987). *Biochemistry* **26**, 8503–8507.

Boyer, P. D. (1989). *FASEB J.* **3**, 2164–2178.

Dunn, S. D., and Futai, M. (1980). *J. Biol. Chem.* **255**, 113–118.

Frasch, W. D., Green, J., Caguiat, J., and Mejia, A. (1989). *J. Biol. Chem.* **264**, 5064–5069.

Futai, M. (1977). *Biochem. Biophys. Res. Commun.* **79**, 1231–1237.

Futai, M., Kanazawa, H., Takeda, K., and Kagawa, Y. (1980). *Biochem. Biophys. Res. Commun.* **96**, 227–234.

Futai, M., Noumi, T., and Maeda, M. (1988). *J. Bioenerg. Biomembr.* **20**, 469–480.

Futai, M., Noumi, T., and Maeda, M. (1989). *Annu. Rev. Biochem.* **58**, 111–136.

Gromet-Elhanan, Z., and Avital, S. (1992). *Biochim. Biophys. Acta.* (in press).

Gromet-Elhanan, Z., and Khananshvili, D. (1984). *Biochemistry* **23**, 1022–1028.

Gromet-Elhanan, Z., Khananshvili, D., Weiss, S., Kanazawa, H., and Futai, M. (1985). *J. Biol. Chem.* **260**, 12635–12640.

Harada, M., Ohta, S., Sato, M., Ito, Y., Kobayashi, Y., Sone, N., Ohta, T., and Kagawa, Y. (1991). *Biochim. Biophys. Acta* **1056**, 279–294.

Harris, D. A., Boork, J., and Baltscheffsky, M. (1985). *Biochemistry* **24**, 3876–3883.

Hisabori, T., Yoshida, M., and Sakurai, H. (1986). *J. Biochem.* **100**, 663–670.

Hsu, S. Y., Senda, M., Kanazawa, H., Tsuchiya, T., and Futai, M. (1984). *Biochemistry* **23**, 988–993.

Issartel, J. P., and Vignais, P. V. (1984). *Biochemistry* **23**, 6591–6595.

Jagendorf, A. T., McCarty, R. E., and Robertson, D. (1991). *Cell Culture and Somatic Genetics of Plants* **7B**, 225–254.

Kagawa, Y., Ohta, S., and Otawara-Hammamoto, Y. (1989). *FEBS Lett.* **249**, 67–69.

Khananshvili, D., and Gromet-Elhanan, Z. (1982). *J. Biol. Chem.* **257**, 11377–11383.

Khananshvili, D., and Gromet-Elhanan, Z. (1984). *FEBS Lett.* **178**, 10–14.

Khananshvili, D., and Gromet-Elhanan, Z. (1985a). *Biochemistry* **24**, 2482–2487.

Khananshvili, D., and Gromet-Elhanan, Z. (1985b). *Proc. Natl. Acad. Sci. USA* **82**, 1886–1890.

Khananshvili, D., and Gromet-Elhanan, Z. (1986). *Biochemistry* **25**, 6139–6144.

McCarty, R. E., and Richter, M. L. (1987). In *Perspectives of Biological Energy Transduction* (Mukohata, Y., Morales, M. F., and Fleisher, S., eds.), Academic Press, Orlando, Florida, pp. 369–375.

Miwa, K., and Yoshida, M. (1989). *Proc. Natl. Acad. Sci. USA* **86**, 6484–6487.

Ohta, S., Tsuboi, M., Oshima, T., Yoshida, M., and Kagawa, Y. (1980). *J. Biochem.* **87**, 1609–1617.

Ohta, S., Yohda, M., Ishizuka, M., Hirata, H., Hammamoto, T., Otawara-Hamamoto, Y., Matsuda, K., and Kagawa, Y. (1988). *Biochim. Biophys. Acta* **933**, 141–155.

Patrie, W. J., and McCarty, R. E. (1984). *J. Biol. Chem.* **259**, 11121–11128.

Penefsky, H. S., and Cross, R. L. (1991). *Adv. Enzymol.* **64**, 173–214.

Philosoph, S., Binder, A., and Gromet-Elhanan, Z. (1977). *J. Biol. Chem.* **252**, 8747–8752.

Richter, M., Gromet-Elhanan, Z., and McCarty, R. E. (1986). *J. Biol. Chem.* **261**, 12109–12113.

Roux-Fromy, M., Newmann, J. M., Berger, A. F., Girault, G., Galmiche, J. M., and Remy, R. (1987). *Biochem. Biophys. Res. Commun.* **144**, 718–725.

Senda, M., Kanazawa, H., Tsuchiya, T., and Futai, M. (1983). *Arch. Biochem. Biophys.* **220**, 398–404.

Senior, A. E. (1990). *Annu. Rev. Biophys. Chem.* **19**, 7–11.

Shapiro, A. B., Gibson, K. D., Scheraga, H. A., and McCarty, R. E. (1991). *J. Biol. Chem.* **266**, 17276–17285.

Steele, J. A., Uchytil, T. F., Durbin, R. D., Bhatnagar, P., and Rich, D. H. (1976). *Proc. Natl. Acad. Sci. USA* **73**, 2245–2248.

Steele, J. A., Uchytil, T. F., and Durbin, R. D. (1978). *Biochim. Biophys. Acta* **504**, 136–141.

Yoshida, M., Sone, H., Hirata, H., and Kagawa, Y. (1977). *J. Biol. Chem.* **252**, 3480–3485.

Ysern, X., Amzel, M., and Pedersen, P. L. (1988). *J. Bioenerg. Biomembr.* **20**, 423–450.